Journal of Food Protection, Vol. 67, No. 10, 2004, Pages 2151–2157

Effectiveness of Cleaners and Sanitizers in Killing *Salmonella* Newport in the Gut of a Free-Living Nematode, *Caenorhabditis elegans*

STEPHEN J. KENNEY,[1] GARY L. ANDERSON,[2] PHILLIP L. WILLIAMS,[2] PATRICIA D. MILLNER,[3] AND LARRY R. BEUCHAT[1]*

[1]*Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797;* [2]*Department of Environmental Health Science, University of Georgia, Athens, Georgia 30602-2102; and* [3]*U.S. Department of Agriculture, Agricultural Research Service, Building 001, Room 140, 10300 Baltimore Avenue, Beltsville, Maryland 20705-2350, USA*

MS 04-62: Received 16 February 2004/Accepted 25 April 2004

ABSTRACT

Caenorhabditis elegans, a free-living nematode found in soil, has been shown to ingest human enteric pathogens, thereby potentially serving as a vector for preharvest contamination of fruits and vegetables. A study was undertaken to evaluate the efficacy of cleaners and sanitizers in killing *Salmonella enterica* serotype Newport in the gut of *C. elegans.* Adult worms were fed nalidixic acid–adapted cells of *Escherichia coli* OP50 (control) or *Salmonella* Newport for 24 h, washed, placed on paper discs, and incubated at temperatures of 4 or 20°C and relative humidities of 33 or 98% for 24 h. Two commercial cleaners (Enforce and K Foam Lo) and four sanitizers (2% acetic acid, 2% lactic acid, Sanova, and chlorine [50 and 200 μg/ml]) were applied to worms for 0, 2, or 10 min. Populations of *E. coli* and *Salmonella* Newport (CFU per worm) in untreated and treated worms were determined by sonicating worms in 0.1% peptone and surface plating suspensions of released cells on tryptic soy agar containing nalidixic acid. Populations of *Salmonella* Newport in worms exposed to 33 or 98% relative humidity at 4°C or 33% relative humidity at 20°C were significantly ($P \leq 0.05$) lower than the number surviving exposure to 98% relative humidity at 20°C. In general, treatment of desiccated worms with cleaners and sanitizers was effective in significantly ($P \leq 0.05$) reducing the number of ingested *Salmonella* Newport. Results indicate that temperature and relative humidity influence the survival of *Salmonella* Newport in the gut of *C. elegans,* and cleaners and sanitizers may not eliminate the pathogen.

Outbreaks of foodborne illness associated with the consumption of raw or minimally processed fruits and vegetables have increased in recent years *(4, 15, 20).* These outbreaks have raised interest in determining mechanisms through which preharvest and postharvest produce becomes contaminated with enteric pathogens.

Application of animal manure to cropland soil as a fertilizer is not an uncommon practice. Human pathogenic bacteria have been isolated from soils to which bovine manure had been applied *(12).* The microbial profile of the soil in which fruits and vegetables are grown or come in contact can have an impact on the postharvest safety and quality of the produce *(5, 16).* This was illustrated in outbreaks of cryptosporidosis and *Escherichia coli* O157:H7 infection associated with cider made in part from apples that had fallen from trees before harvest *(10, 11, 19).* Runoff water from cattle pastureland may have contaminated the apples. The skin of tomatoes in contact with soil containing *Salmonella* can become contaminated with the pathogen *(14).*

Large microbial populations in the soil matrices, such as those amended with manure, have been reported to attract free-living nematodes *(21). Caenorhabditis elegans,* a free-living, microbivorous nematode found in the soil of temperate regions, has been reported to ingest *E. coli* O157:H7, *Listeria monocytogenes (2, 8),* several serotypes of *Salmonella enterica (1, 8, 17), Bacillus cereus (2),* and *Staphylococcus aureus (24).* Depending on environmental conditions, bacteria may persist within the gut of *C. elegans* for several days after consumption *(1, 17).* Releases of pathogens as a result of rupturing of the cuticle or defecation are ways that infected nematodes can contaminate the soil environment. *C. elegans* can transport ingested pathogens to the surface tissues of produce in contact with soil. *Salmonella enterica* serotype Poona, placed in soil 1 or 3 cm away from cantaloupe rind, was detected on rind in contact with the soil containing *C. elegans* within a shorter time compared to rind on soil void of the nematode *(8).*

In general, free-living nematodes are sensitive to desiccation. Worms in soil, on the surface of root crops, or on aerial parts of plants as a result of postharvest handling can quickly become desiccated. Carcasses of desiccated worms may harbor viable bacteria that were ingested by the worm prior to death. The cuticle of a worm may provide a physical barrier between bacteria resident in the gut and commercial cleaners and sanitizers used to remove soil or decontaminate equipment and produce. Salmonellae *(7, 9)* and *Shigella sonnei (9)* have been reported to survive sanitizer treatments in nematodes. Bacterial cells surviving environmental stresses imposed by desiccation, commercial clean-

* Author for correspondence. Tel: 770-412-4740; Fax: 770-229-3216; E-mail: lbeuchat@uga.edu.

ers, and sanitizers may remain on the produce until ingested by the consumer.

Commercial cleaners and sanitizers used by the produce industry may contain surfactants to aid in the release of microorganisms, and perhaps also nematodes, from the surface of produce. Nematodes may subsequently become resident in or on produce contact areas such as water baths, belts, tables, and sorters in processing facilities. Pathogens from a single worm released onto processing equipment could theoretically release ingested cells of a pathogen and contaminate large amounts of produce. The plausibility of this series of events happening on a commercial level can be more easily assessed if the effectiveness of cleaners and sanitizers in killing pathogens ingested by nematodes is known.

A study was undertaken to determine the effectiveness of two commercial cleaners and four sanitizers in killing *E. coli* OP50 and *Salmonella* Newport in the gut of *C. elegans.* The effectiveness of these treatments in killing planktonic cells of *E. coli* OP50 and *Salmonella* Newport was also evaluated.

MATERIALS AND METHODS

Maintenance of *C. elegans.* A wild-type (N2) strain of *C. elegans* was used. Worms were maintained on K agar (pH 6.5), which contains (per liter of deionized water): potassium chloride (2.36 g), sodium chloride (3.0 g), Bacto Peptone (2.5 g; BBL/Difco, Becton Dickinson, Sparks, Md.), and agar (17.0 g) *(26). E. coli* OP50, an avirulent strain routinely used as a feed source for *C. elegans,* was cultured at 37°C for 24 h in OP50 broth, which contains (per liter of deionized water): sodium chloride (5.0 g) and Bacto Peptone (10.0 g) *(6).* The K agar was surface inoculated with 0.1 ml of a 24-h culture of *E. coli* OP50 and incubated at 37°C for 24 h to establish confluent growth. Approximately 50 adult worms were deposited on the surface of K agar and incubated at 20°C for up to 3 days prior to transferring worms to a fresh K agar plate with a 24-h lawn of *E. coli* OP50 or *Salmonella* Newport.

Preparation of *C. elegans* for enteric pathogen assay. Three-day-old worms were transferred to a fresh K agar plate on which a 24-h lawn of *E. coli* O157:H7 or *Salmonella* Newport had grown. The surface of 10 K agar plates, each containing 500 to 1,000 eggs and 30 to 50 adult worms, was washed by depositing 5 ml of sterile K broth *(27)* and gently rubbing with a sterile bent glass rod. The suspended eggs and worms were aseptically transferred to a sterile 15-ml centrifuge tube. The wash and transfer procedure was repeated to enable efficient harvesting of eggs. Eggs and worms were collected by centrifugation (500 × *g,* 2 min), and the supernatant was removed using a pipette. Worms and eggs in a pellet from pooled suspensions were resuspended in 10 ml of 0.013 M NaOH solution containing 1% NaOCl (pH 12.97) and incubated at 20°C for 15 min to kill all life cycle forms of the worm except the eggs. The suspension was centrifuged (500 × *g,* 2 min), and the supernatant was removed. Worms and eggs in the pellet were resuspended in 10 ml of K medium and centrifuged again. The supernatant was removed, and the eggs and dead worms were resuspended in K medium. The suspension (0.1 ml containing 400 to 600 eggs) was deposited on the surface of a K agar plate on which a lawn of *E. coli* OP50 had formed and was then incubated at 20°C for 3 days. This procedure ensured that all worms used in assays were of the same age. Adult worms were used in assays to confirm that they will ingest pathogenic bacteria and, once ingested, to determine the efficacy of cleaners and sanitizers in killing *E. coli* OP50 and *Salmonella* Newport in the gut as affected by temperature and relative humidity.

TABLE 1. *Treatments evaluated for killing of* Escherichia coli *OP50 and* Salmonella *Newport ingested by* Caenorhabditis elegans

Code	Treatment	Concn	pH[a]
Control			
	Distilled water	100%	7.8
A	Peptone water	0.1%	6.1
Cleaner			
B	K Foam Lo	100%[b]	11.1
C	Enforce	100%[b]	12.3
Sanitizer			
D	Sanova	1,200 μg/ml	2.4
E	Acetic acid	2%	2.4
F	Lactic acid	2%	2.0
G	NaOCl	50 μg/ml	6.9
H	NaOCl	200 μg/ml	6.9

[a] pH of a working concentration of the treatment solution at 20°C.
[b] 100% of manufacturers' recommended working concentration.

Preparation of cleaner and sanitizer solutions. Seven chemical solutions used or having potential for use as cleaners or sanitizers in the produce industry were evaluated for their effectiveness in killing *E. coli* OP50 and *Salmonella* Newport ingested by *C. elegans* (Table 1). K Foam Lo (CK Enterprises, Inc., Lee's Summit, Mo.), an alkaline ethylene glycol monobutyl ether solution, and Enforce (Ecolab, St. Paul, Minn.), a self-foaming chlorinated alkaline cleaner, were tested at 100% of the manufacturers' recommended working concentration. Sanova, an acidified sodium chlorite sanitizer (Alcide Corporation, Redmond, Wash.), and acetic and lactic acids, each at 2%, were evaluated. Chlorine solutions were prepared by combining sodium hypochlorite (Aldrich Chemical Co., Inc., Milwaukee, Wis.) with 0.05 M potassium phosphate buffer (pH 6.8). The concentration of free chlorine was measured by using a DR/820 colorimeter (Hach, Loveland, Colo.). Sterile distilled water and 0.1% peptone water were used as controls. All chemical treatment solutions were used within 30 min of preparation.

Bacteria used and preparation of worms for desiccation assays. *E. coli* OP50 (control) and a multidrug resistant strain of *Salmonella* Newport were evaluated. Both strains were adapted to grow in tryptic soy broth (TSB, pH 7.3; BBL/Difco) supplemented with nalidixic acid (50 μg/ml) (TSBN). Nalidixic acid–adapted *E. coli* OP50 and *Salmonella* Newport were used to facilitate their detection on tryptic soy agar (BBL/Difco) supplemented with nalidixic acid (50 μg/ml) (TSAN). Sensitive *E. coli* OP50 cells remaining on the surface or in the gut of the worm as a result of feeding before exposure to nalidixic acid–adapted cells would not be expected to grow on TSAN, but adapted cells would form colonies. Nalidixic acid–adapted cells were grown in 10 ml of TSBN at 37°C for 24 h. *E. coli* OP50 and *Salmonella* Newport were transferred twice to 10 ml of TSBN by loop inocula at successive 24-h intervals. Inoculum (0.1 ml of a 24-h culture) was surface plated on K agar supplemented with 50 μg nalidixic acid per ml (KN agar) and incubated at 37°C for 24 h to produce a lawn of cells. Three-day-old adult worms from a synchronized

culture grown on K agar with lawns of nalidixic acid–sensitive *E. coli* OP50 were placed on the surface of KN agar plates individually containing lawns of *E. coli* OP50 or *Salmonella* Newport and allowed to feed on cells for 24 h at 20°C. K medium (10 ml) was deposited on the surface of plates, and worms and bacteria were suspended using a sterile glass rod. Suspensions were placed in sterile 15-ml centrifuge tubes, centrifuged (500 × *g*, 2 min), resuspended in K medium, and centrifuged again. Washed worms (100 to 200 worms) in 0.5 ml of K medium were placed on the surface of uninoculated K agar, and the K medium was allowed to absorb into agar at 20°C for 1 h. These worms were used in assays to test the effectiveness of cleaners and sanitizers in killing ingested *E. coli* OP50 and *Salmonella* Newport.

Atmospheric desiccation of worms and treatment with cleaners and sanitizers. Relative humidities of 33 or 98% inside 1.7-liter containers were achieved by depositing 300 ml of saturated solutions of magnesium chloride or disodium phosphate, respectively, beneath an elevated surface of a platform on which inoculated paper discs (6 mm in diameter) would be eventually placed. Ten worms were removed from the surface of K agar using a platinum wire, suspended in 5 μl of sterile 0.1% peptone water, and inoculated onto each disc. Initial populations of test bacteria were determined by analyzing suspensions of worms before inoculating discs. The inoculated discs were placed in containers with 33 or 98% relative humidity and sealed by applying a lid. Worms were incubated at 4 or 20°C for 24 h before applying a cleaner or sanitizer treatment and analyzing for populations of test bacteria. Each disc was aseptically transferred to a sterile 15-ml centrifuge tube containing 5 ml of sterile distilled water (control), sterile 0.1% peptone water (control), cleaner, or sanitizer at 20°C (Table 1). After 2 or 10 min, 5 ml of Dey-Engley neutralizing broth (BBL/Difco) was added to each tube. The disc and water or treatment solution were sonicated (Sonifier 450, Branson Ultrasonics, Danbury, Conn.) using a duty cycle of 25% for 25 s at 21°C to rupture the cuticle of *C. elegans* and release ingested bacteria. Sonicate was serially diluted in sterile 0.1% peptone and surface plated (0.1 ml in duplicate) on TSAN containing 0.1% sodium pyruvate (TSANP). Plates were incubated at 37°C for 24 h before presumptive colonies of *E. coli* OP50 or *Salmonella* Newport were counted. Random colonies were confirmed using API 20E miniaturized diagnostic kits (bioMérieux Vitek, Inc., Hazelwood, Mo.) and the *Salmonella* latex agglutination assay (FT 0203A; Oxoid Ltd., Basingstoke, Hampshire, UK).

Treatment of planktonic cells with cleaners and sanitizers. *E. coli* OP50 and *Salmonella* Newport were grown in 10 ml of TSBN at 37°C for 24 h and then incubated at 4 or 20°C for an additional 24 h to approximate environmental conditions that cells ingested by *C. elegans* were subjected to in studies involving desiccated worms. Cell suspensions were centrifuged at 2,000 × *g* for 10 min. Supernatants were decanted, and cells were resuspended in 10 ml of sterile 0.1% peptone water. Centrifugation and resuspension of cells were repeated two times before treating with distilled water (control), cleaners, or sanitizers as described above, with the exception that sonication was omitted. Undiluted and diluted suspensions of control and treated cells were spread plated on TSANP agar and incubated at 37°C for 24 h. Presumptive colonies of *E. coli* OP50 and *Salmonella* Newport were enumerated and confirmed as described above.

Statistical analysis. Each experiment was replicated three times. Data were analyzed using the General Linear Models procedure of SAS (Statistical Analysis Systems Institute, Cary, N.C.). Significant differences ($P \leq 0.05$) between mean values were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Effectiveness of cleaners and sanitizers in killing *E. coli* OP50 and *Salmonella* Newport ingested by *C. elegans*. Mean populations of *E. coli* OP50 and *Salmonella* Newport recovered from untreated *C. elegans* and from worms treated in distilled water (control), cleaners, or sanitizers are shown in Table 2. With the exception of worms that had fed on *Salmonella* Newport and were incubated at 33% relative humidity before treatment for 10 min, the initial number of *E. coli* OP50 and *Salmonella* Newport recovered from worms before incubation at 4°C was not significantly ($P > 0.05$) different from the number recovered from worms treated with distilled water or peptone water (treatment A). Neither bacterium would be expected to grow at 4°C or 33% relative humidity, so the increase in the population of *Salmonella* Newport is considered an aberrant data point.

Significant increases ($P \leq 0.05$) in *E. coli* OP50 and *Salmonella* Newport populations occurred in worms incubated at 20 and 98% relative humidity, as evidenced by numbers of both bacteria recovered from worms treated with water or 0.1% peptone for 10 min. Bacteria present in the gut of dead worms apparently utilized nutrients released by the autolysis. This is supported by a study that showed bacterial populations increasing in carcasses of *C. elegans* incubated at 37°C on nutrient-void Bacto agar *(17)*. Degradative enzyme activity would be higher at 20°C than at 4°C. Bacterial cells in worms incubated at 20°C and 33% relative humidity may have been stressed by a reduction in water activity or are more sensitive to the degradative enzymes promoting autolysis, resulting in their death or inability to grow. Incubation at 20°C and 98% relative humidity would not be expected to inhibit the growth of *E. coli* OP50 or *Salmonella* Newport.

Overall, K Foam Lo (treatment B) was the least effective in killing *E. coli* OP50 and *Salmonella* Newport in *C. elegans*. Up to a 1.12-log CFU per worm reduction in *E. coli* OP50, compared to the initial population, was observed in worms immersed in this cleaner. Treatment of worms that had been held at 4°C and 33% relative humidity for 10 min or worms held at 4°C and 98% relative humidity for 2 or 10 min with K Foam Lo caused significant reductions in populations of *E. coli* OP50 compared to treatment with distilled water or 0.1% peptone (treatment A). K Foam Lo was ineffective in killing *E. coli* OP50 in worms that had been held at 20°C and 33 or 98% relative humidity. In a study evaluating the lethality of seven commercially available alkaline cleaners in killing *E. coli* O157:H7, it was demonstrated that K Foam Lo was the least effective *(23)*. Considering all combinations of incubation temperature, relative humidity, and treatment time, except for worms incubated at 4°C and 33% relative humidity and treated for 2 min, mean populations of *Salmonella* Newport were significantly reduced ($P \leq 0.05$) by up to 4.92 log CFU per worm compared to mean populations detected in worms treated with distilled water or peptone water.

TABLE 2. *Populations of* Escherichia coli *OP50 and* Salmonella *Newport recovered from* Caenorhabditis elegans *incubated at 4 or 20°C and relative humidities of 33 or 98% for 24 h and then treated with commercial cleaners and sanitizers*

Bacterium	Temp (°C)	Relative humidity (%)	Treatment time (min)	Populations (log CFU/worm)[a]									
					Treatment[b]								
				Initial	Distilled water	A	B	C	D	E	F	G	H
E. coli OP50	4	33	2	1.53 A	1.12 A	1.59 A	1.30 A	0.16 A	<0.04 B (0)	<0.04 B (0)	0.01 B	0.01 B	<0.04 B (0)
			10	1.53 A	1.42 A	1.45 A	0.89 B	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	0.01 C	<0.04 C (0)
		98	2	1.53 B	1.82 A	2.01 A	1.52 B	0.01 C	<0.04 C (1)	2.65 A	<0.04 C (0)	0.23 C	<0.04 C (0)
			10	1.53 A	1.81 A	1.83 A	0.45 B	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	0.01 C	<0.04 C (0)	<0.04 C (0)
	20	33	2	1.53 B	0.47 C	0.60 C	0.41 CD	0.01 D	<0.04 D (0)	<0.04 D (0)	2.75 A	<0.04 D (0)	<0.04 D (0)
			10	1.53 A	0.83 B	0.67 B	0.59 B	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)
		98	2	1.53 B	1.00 B	1.18 B	1.03 B	<0.04 C (0)	<0.04 C (0)	2.86 A	<0.04 B (0)	<0.04 C (0)	<0.04 C (0)
			10	1.53 B	2.44 A	2.47 A	2.38 A	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)
Salmonella Newport	4	33	2	1.55 AB	1.52 AB	2.76 A	0.17 B	<0.04 B (0)	0.01 B	0.77 B	<0.04 B (0)	0.55 B	1.35 AB
			10	1.55 B	2.15 A	2.26 A	0.01 C	<0.04 C (0)	<0.04 C (0)	0.21 C	<0.04 C (0)	0.19 C	<0.04 C (0)
		98	2	1.55 B	1.98 B	2.03 B	0.43 C	0.01 C	<0.04 C (1)	0.21 C	<0.04 C (0)	0.40 C	0.17 C
			10	1.55 A	1.97 A	1.95 A	0.01 C	<0.04 C (0)	<0.04 C (0)	0.11 C	<0.04 C (0)	0.03 C	<0.04 C (1)
	20	33	2	1.55 A	0.98 AB	1.00 AB	0.10 C	<0.04 C (0)	<0.04 C (0)	0.55 CB	0.03 C	0.15 C	0.79 CB
			10	1.55 A	1.12 AB	1.17 AB	0.01 C	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	<0.04 C (0)	0.93 ABC	<0.04 C (0)
		98	2	1.55 CB	4.44 A	4.62 A	0.42 D	<0.04 D (0)	0.01 D (1)	2.35 B	<0.04 D (0)	2.11 CB	1.91 CB
			10	1.55 B	4.57 A	4.93 A	0.01 D	<0.04 D (0)	<0.04 D (0)	1.33 BC	<0.04 D (0)	0.74 BCD	0.48 CD

[a] Mean values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$); the minimum detection limit was 0.04 CFU per worm. Numbers in parentheses following values indicate the number of positive samples of three samples analyzed.

[b] Refer to Table 1 for list of treatments.

Treatments C (Enforce), D (Sanova), and F (2% lactic acid) were the most effective among test cleaners and sanitizers in eliminating test microorganisms ingested by *C. elegans.* Enforce is a commercially available cleaner and has the highest pH (12.3) of all products evaluated. Sanova, an acidified sodium chlorite–based sanitizer with a pH of 2.4, had the same pH as 2% acetic acid (treatment E); however, Sanova was much more effective in killing ingested bacterial cells. This indicates that factors other than pH play a role in the lethality of cleaners and sanitizers to *E. coli* OP50 and *Salmonella* Newport lodged in the gut of *C. elegans. C. elegans* can survive in a pH range of 3.2 to 11.8 for at least 96 h *(18).*

Two organic acids (treatments E and F) were evaluated in this study. Treatment with 2% acetic acid (treatment E) reduced the number of *Salmonella* Newport to a level below the detection limit (0.04 log CFU per worm) in one of eight treatment combinations compared to seven of eight treatment combinations for 2% lactic acid (treatment F). This agrees with results reported by Caldwell et al. *(7)* in which higher initial populations of ingested *Salmonella* Poona were reduced by up to 1.61 log CFU per worm treated with 2% acetic acid compared to reductions of 5.32 log CFU per worm when treated with 2% lactic acid. Other studies have shown acetic acid to be more effective than lactic acid in killing *E. coli* O157:H7 *(13, 22)* and *Shigella flexneri (25).* At a working concentration of 2%, the pH of lactic acid (2.0) is lower than that of acetic acid (2.4). The lower pH may be partially responsible for the increased effectiveness of lactic acid in killing *E. coli* OP50 and salmonellae in the gut of *C. elegans.*

Solutions of NaOCl containing free chlorine at 50 μg/ml (treatment G) and 200 μg/ml (treatment H) were more effective in reducing *E. coli* OP50 populations than *Salmonella* Newport populations. Compared to the number of *E. coli* OP50 recovered from worms treated with water or peptone, treatment with both concentrations of chlorine caused significant ($P \leq 0.05$) reductions in counts, regardless of previous desiccation conditions or treatment time. Populations were reduced to undetectable levels in worms that had been subjected to five of eight temperature × relative humidity × treatment time combinations, regardless of the concentration of chlorine in the treatment solution. In contrast, *Salmonella* Newport was recovered from desiccated worms that had been exposed to all combinations of incubation temperature and relative humidity after treatment with 50 or 200 μg of chlorine per ml for 2 min. With one exception (20°C, 33% relative humidity), compared to treatment with water or peptone for 10 min, significant reductions in populations of *Salmonella* Newport were achieved by treating worms with both concentrations of chlorine for 10 min. In the presence of organic matter, the efficacy of chlorine in killing bacteria is greatly diminished. The treatment solutions in which desiccated worms were immersed contain little or no organic matter other than that in the worms and the bacteria within them. This study represents a best-case scenario in terms of testing the effectiveness of chlorine in killing bacteria ingested by worms. The ability of chlorine to kill ingested bacteria would be further diminished if worms were in environments such as produce-processing facilities or on the surface of produce, because of the presence of large amounts of organic matter in wash waters and in produce tissues. For disinfection of produce using chlorine, contact times of no more than 1 to 2 min are commonly used *(3).* A chlorine concentration of 200 μg/ml is the upper limit used by the produce industry, but most processors use substantially lower concentrations. For chlorine to be effective at killing bacteria located in the gut of *C. elegans* and perhaps other free-living nematodes, the concentration of free chlorine in wash water would have to be closely monitored, and contact times might have to be increased. To be effective in eliminating bacteria from the gut of *C. elegans,* treatments must penetrate the cuticle of the worm or otherwise disrupt the integrity of the cuticle and come into contact with the target cells. Enforce and Sanova both contain bactericidal constituents that apparently have a synergistic effect with pH. The cuticular permeability of desiccated *C. elegans* may increase as the pH of the environment deviates from neutral, allowing bactericides in cleaners and sanitizers to penetrate the cuticle and come in contact with ingested cells.

Effectiveness of cleaners and sanitizers in killing planktonic *E. coli* OP50 and *Salmonella* Newport cells. Mean populations of planktonic cells of *E. coli* OP50 and *Salmonella* Newport that were recovered after treatment with distilled water (control), 0.1% peptone, cleaners, and sanitizers are shown in Table 3. Initial mean populations of test microorganisms were not significantly ($P > 0.05$) influenced by treatment of cells that had been held at 4 or 20°C for 24 h in distilled water or peptone (treatment A).

E. coli OP50 and *Salmonella* Newport were not detected by direct plating or enrichment after treatment with Sanova (treatment D), 50 μg of chlorine per ml (treatment G), or 200 μg of chlorine per ml (treatment H). The two cleaners, K Foam Lo (treatment B) and Enforce (treatment C), were less effective in killing the two microorganisms. Considering the incubation temperature preceding treatment as well as the treatment time, K Foam Lo significantly ($P \leq 0.05$) reduced populations of *E. coli* OP50 and *Salmonella* Newport by up to 4.27 and 4.25 log CFU per worm, respectively, compared to reductions caused by treating cells with 0.1% peptone. While reductions were significant, both test microorganisms were detected by direct plating. In general, compared to reductions in populations caused by K Foam Lo, significantly greater reductions of *E. coli* OP50 were achieved by treating cells with Enforce.

Treatment with 2% lactic acid (treatment F) was significantly ($P \leq 0.05$) more effective than treatment with 2% acetic acid (treatment E) in reducing populations of *E. coli* OP50 and *Salmonella* Newport. Acetic acid was the least effective chemical treatment evaluated in killing planktonic cells of both test microorganisms.

Treatment with cleaners and sanitizers may be more effective in killing or releasing ingested bacterial cells from the gut of desiccated worms than from live worms. Placement of desiccated worms in an environment with a higher moisture content, relative to the worms, would result in

TABLE 3. *Populations of planktonic* Escherichia coli *OP50 and* Salmonella *Newport recovered from cells incubated at 4 or 20°C for 24 h and then treated with commercial cleaners and sanitizers*

Bacterium	Temp (°C)	Treatment time (min)	Populations (log CFU/worm)[a]								
			Treatment[b]								
			Initial	Distilled water	A	B	C	D	E	F	G	H
E. coli OP50	4	2	5.46 A	5.17 A	5.25 A	2.93 C	0.54 E	<0.04 E (0)	4.41 B	2.00 D	<0.04 E (0)	<0.04 E (0)
		10	5.46 A	5.15 A	5.22 A	0.95 C	<0.04 D (0)	<0.04 D (0)	4.33 B	1.02 CD	<0.04 E (0)	<0.04 E (0)
	20	2	5.46 A	5.22 A	5.21 A	3.37 C	1.22 E	<0.04 F (0)	4.38 B	1.93 D	<0.04 F (0)	<0.04 F (0)
		10	5.46 A	5.27 A	5.14 A	1.52 C	0.73 DE	<0.04 E (0)	4.41 B	1.02 CD	<0.04 E (0)	<0.04 E (0)
Salmonella Newport	4	2	4.47 A	4.64 A	4.55 A	2.63 C	<0.04 B (0)	<0.04 D (0)	4.23 B	2.41 C	<0.04 D (0)	<0.04 D (0)
		10	4.47 A	4.66 B	4.63 A	0.38 B	<0.04 B (0)	<0.04 B (0)	4.11 A	0.43 B	<0.04 B (0)	<0.04 B (0)
	20	2	4.47 AB	4.62 A	4.58 A	2.29 C	0.53 D	<0.04 E (0)	4.17 B	2.16 C	<0.04 E (0)	<0.04 E (0)
		10	4.47 A	4.77 A	4.63 A	0.63 CD	0.91 BC	<0.04 D (0)	4.21 A	1.50 B	<0.04 D (0)	<0.04 D (0)

[a] Mean values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$); the minimum detection limit was 0.04 CFU per worm. Numbers in parentheses following values indicate the number of positive samples of three samples analyzed.

[b] Refer to Table 1 for list of treatments.

absorption of water. Bactericidal compounds in the water would come in contact with bacterial cells in the gut of the worm. Worms not desiccated before exposure to cleaners or sanitizers would absorb less water—and therefore, lesser amounts of bactericidal constituents. During the process of desiccation and rehydration, the cuticular structure of the nematode may become compromised, thereby resulting in increased exposure of the gut contents to cleaners and sanitizers and a higher level of lethality compared to that achieved by treating worms that are not desiccated.

In summary, none of the commercial cleaners and sanitizers evaluated were effective in killing all cells of *Salmonella* Newport ingested by *C. elegans* using all test conditions. The use of cleaners and sanitizers in combination with other treatments to kill bacteria ingested by nematodes is an important step in reducing pathogens on produce contact surfaces and on raw or minimally processed fruits and vegetables destined for the retail market.

ACKNOWLEDGMENTS

This study was supported in part by the USDA-ARS National Alliance for Food Safety and Security. The *C. elegans* strain used in this work was provided by the Caenorhabditis Genetic Center, which is funded by the NIH National Center for Research Resources.

REFERENCES

1. Aballay, A., P. Yorgey, and F. M. Ausubel. 2000. *Salmonella enterica typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans. Curr. Biol.* 10:1539–1542.
2. Anderson, G. L., K. N. Caldwell, L. R. Beuchat, and P. L. Williams. 2003. Interaction of a free-living soil nematode, *Caenorhabditis elegans*, with surrogates of foodborne pathogenic bacteria. *J. Food Prot.* 66:1543–1549.
3. Beuchat, L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. WHO/FSF.FOS/98.2. World Health Organization, Food Safety Unit, Geneva.
4. Beuchat, L. R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect.* 4:413–423.
5. Brackett, R. E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharv. Biol. Technol.* 15:305–311.
6. Brenner, S. 1974. The genetics of *Caenorhabditis elegans. Genetics.* 77:71–94.
7. Caldwell, K. N., B. B. Adler, G. L. Anderson, P. L. Williams, and L. R. Beuchat. 2003. Ingestion of *Salmonella enterica enterica* serotype Poona by a free-living nematode, *Caenorhabditis elegans*, and protection against inactivation by produce sanitizers. *Appl. Environ. Microbiol.* 69:4103–4110.
8. Caldwell, K. N., G. L. Anderson, P. L. Williams, and L. R. Beuchat. 2003. Attraction of a free-living nematode, *Caenorhabditis elegans*, to foodborne pathogenic bacteria and its potential as a vector of *Salmonella* Poona for preharvest contamination of cantaloupe. *J. Food Prot.* 66:1964–1971.
9. Chang, S. L., G. Berg, N. A. Clarke, and P. W. Kabler. 1960. Survival and protection against chlorination of human enteric pathogens in free-living nematodes isolated from water supplies. *Am. J. Trop. Med. Hyg.* 9:136–142.
10. Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. *Morb. Mortal. Wkly. Rep.* 46:4–8.
11. Cody, S. H., M. K. Glynn, J. A. Farrar, K. L. Cairns, P. M. Griffin, J. Kobayashi, M. Fyfe, R. Hoffman, A. S. King, and J. J. Lewis. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Ann. Intern. Med.* 130:202–209.
12. de Freitas, J. R., J. J. Schoenau, S. M. Boyetchko, and S. A. Cyrenne.

2003. Soil microbial populations, community composition, and activity as affected by repeated applications of hog and cattle manure in eastern Saskatchewan. *Can. J. Microbiol.* 49:538–548.
13. Deng, Y., J.-H. Ryu, and L. R. Beuchat. 1999. Tolerance of acid-adapted and non-adapted *Escherichia coli* O157:H7 cells to reduced pH as affected by type of acidulant. *J. Appl. Microbiol.* 86:203–210.
14. Guo, X., J. Chen, R. E. Brackett, and L. R. Beuchat. 2002. Survival of *Salmonella* on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil. *J. Food Prot.* 65:274–279.
15. Institute of Food Technologists/Food and Drug Administration of the United States Department of Health and Human Services (IFT/FDA). 2001. Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. IFT/FDA Contract 223-98-2333. Task Order 3. Institute of Food Technologists, Chicago, Ill.
16. Jay, J. M. 2000. Modern food microbiology, 6th ed. Aspen Publishers, Inc., Gaithersburg, Md.
17. Kenney, S. J., G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat. Persistence of *Escherichia coli* O157:H7, *Salmonella enterica* serotype Newport, and *Salmonella enterica* serotype Poona in the gut of a free-living nematode, *Caenorhabditis elegans,* and transmission to progeny and uninfected worms. Submitted for publication.
18. Khanna, N., C. P. Cressman III, C. P. Tatara, and P. L. Williams. 1997. Tolerance of the nematode *Caenorhabditis elegans* to pH, salinity, and hardness in aquatic media. *Arch. Environ. Contam. Toxicol.* 32:110–114.
19. Millard, P. G., K. F. Gensheimer, D. G. Addiss, D. M. Sosin, G. A. Beckett, A. Houck-Jankoski, and A. Hudson. 1994. An outbreak of cryptosporidiosis from fresh-pressed apple cider. *J. Am. Med. Assoc.* 272:1592–1596.
20. National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 10:117–143.
21. Opperman, M. H., M. Wood, P. J. Harris, and C. P. Cherrett. 1993. Nematode and nitrate dynamics in soils treated with cattle slurry. *Soil Biol. Biochem.* 25:19–24.
22. Ryu, J.-H., and L. R. Beuchat. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. *Int. J. Food Microbiol.* 45:185–193.
23. Sharma, M., and L. R. Beuchat. 2004. Sensitivity of *Escherichia coli* O157:H7 to commercially available alkaline cleaners and subsequent resistance to heat and sanitizers. *Appl. Environ. Microbiol.* 70:1795–1803.
24. Sifri, C. D., J. Begun, F. M. Ausubel, and S. B. Calderwood. 2003. *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. *Infect. Immun.* 71:2208–2217.
25. Tetteh, G. L., and L. R. Beuchat. 2001. Sensitivity of acid-adapted and acid-shocked *Shigella flexneri* to reduced pH achieved with acetic, lactic, and propionic acids. *J. Food Prot.* 64:975–981.
26. Williams, P. L., and D. Dusenbery. 1988. Using the nematode, *Caenorhabditis elegans,* to predict mammalian acute lethality to metallic salts. *Toxicol. Ind. Health* 4:469–478.
27. Williams, P. L., and D. Dusenbery. 1990. Acute toxicity testing using the nematode, *Caenorhabditis elegans. Environ. Toxicol. Chem.* 9:1285–1290.